**Picture Legends**

1. **Query Lab**: Scheme for progression of pre-mRNA splicing, highlighting the different conformations required for the first and second catalytic steps; modulation of transition between the two catalytic steps by spliceosomal mutations results in altered splicing fidelity and splice site choice.

2. **Fyodorov & Skoultchi Labs**: Drosophila melanogaster, a model system to study biochemistry and genetics of chromatin.

3. **Frenette Lab**: A whole-mount images of the bone marrow niche with 3D reconstruction. Arrowheads denote hematopoietic stem cells.

4. **Schildkraut Lab**: Fluorescent antibodies showing replication fork direction in single DNA molecules labeled with halogenated nucleotides.

5. **Skoultchi Lab**: ChIP-Seq – Chromatin immunoprecipitation followed by massive parallel sequencing reveals differences in the DNA binding patterns of transcription factor PU.1 in normal red blood cells (ES-EP) and malignant erythroleukemia cells (MEL).

6. **Kitsis Lab**: Cell death. Healthy (left) and dying (right) HEK293 cells. Blue - Hoechst 33342 staining of nuclei. Red-tetramethyl rhodamine ethyl ester reflecting electrical potential difference across the inner mitochondrial membrane.

7. **Warner Lab**: The structure of a yeast ribosome.

8. **Ye Lab**: Immunofluorescence staining reveals that transcription factors BCL6 (red) and STAT3 (green) are expressed in separate populations of B cells within the germinal center, a dynamic microenvironment critical for T-cell dependent antibody response.

9. **Kielian lab**: Alphavirus infection induces the formation of actin and tubulin-positive intercellular extensions that emanate from infected cells and form stable contacts with neighboring cells, mediating cell-cell transmission of infection. This figure is a confocal image of alphavirus-infected Vero cells, with the red channel showing the virus glycoproteins and the green channel showing phalloidin staining of F-actin.

10. **Stanley Lab**: The Notch ligand Dll3 is upregulated in mid-hind brain of E8.5 mouse embryos lacking O-fucose glycans on Notch receptors.

11. **Kielian Lab**: The structure of the alphavirus membrane fusion protein, which mediates virus infection of host cells.

12. **Edelmann Lab**: Lgr5+ and Paneth cells form a stem cell niche in MMR-deficient intestinal tumors
Welcome to the Albert Einstein College of Medicine and the Department of Cell Biology. Our department is focused on molecular mechanisms in many important areas of cell biology, ranging from stem cells to viruses, DNA replication to RNA processing, gene expression to immunology, glycobiology to cancer. We share many common interests and enjoy an interactive and scientifically stimulating atmosphere that makes the Cell Biology Department a great place to work.

Graduate students in Cell Biology participate in a variety of departmental activities. Note that these are currently online get togethers during the pandemic. The department meets every Friday for a “work-in-progress” seminar in which post-doctoral fellows and graduate students describe the progress of their current research and discuss future directions. The department hosts a bi-weekly seminar program of invited outside speakers, with many opportunities for students and postdocs to meet the speaker for discussion and lunch. There is a departmental journal club series in which students present original articles and discuss over dinner. A Friday afternoon get-together encourages scientific interactions as well as social connections. Every few years, our departmental retreat takes us all to the seashore or mountains for a chance to talk about the big picture of our research, to enjoy poster presentations from students and postdocs, and to try to solve the zany puzzles organized by the skit committee.

On the following pages you will find information about the research programs of the individual faculty members, as well as listings of the current students and postdocs in the department. You can also find out more about the department on our web page at http://www.einstein.yu.edu/cellbiology. Feel free to contact any of us for further discussions.

Enjoy your first year!
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Lindsay LaFave
Satish Nandakumar
Charles Query
Matthew Scharff
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Robert Singer
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# Predoctoral Fellows

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07/13/2021
CANCER DORMANCY: UNDERSTANDING THE BIOLOGY OF METASTASIS

The major challenge faced by physicians is the prevention and treatment of metastasis, the main reason for cancer mortality. Often, cancer patients presumed cured after primary tumor removal and therapy, can carry non-proliferating ‘dormant’ disseminated cancer cells (DCCs) for years before reactivating to form incurable metastasis. In addition, DCCs show resistance to standard treatments by reprogramming themselves in a niche-dependent manner. Our lab specifically focused on the dormant nature of minimal residual disease in cancer by understanding the biology of dormant DCCs and their reactivation, to target them and prevent relapse. We focus on identifying the mechanisms of microenvironmental cues and cross-talk with DCCs, epigenetic and immune regulation of DCCs, and developing therapeutic approaches to target DCCs clinically.

1. Mechanisms of early dissemination, dormancy and metastasis. We explored why in many patients metastasis remain dormant in the bone marrow. We found that the bone marrow contains high levels of TGFβ2, which induced dormancy via TGFβ-RI/RII/RIII complexes and p38 signaling. Using mouse genetics, we showed that a key source of TGFβ2 is the NG2+/Nestin+ mesenchymal stem cells (MSCs) that induce hematopoietic stem cell dormancy and self-renewal and also proved that these MSCs induce and maintain dormancy of breast cancer DCCs in the bone marrow. Analysis of the transcriptional and epigenetic mechanisms active in dormant DCCs pinpointed retinoic acid as a micro-environmental pro-dormancy cue. We found that the orphan nuclear receptor NR2F1, which regulates lineage commitment and is silenced in human tumors, is spontaneously upregulated in solitary dormant tumor cells. Retinoic acid induces TGFβ2, NR2F1 expression and dormancy of DCCs. We found that a transient treatment with low dose of 5-azacytidine, followed by all-trans retinoic acid, restored the NR2F1-driven program and long-term in vivo quiescence of previously malignant cells. Our work revealed markers that predict for longer metastasis-free periods in ER+ breast cancer patients. These signatures can be found in single DCCs from prostate cancer patients in clinical dormancy for up to 18 years and not in DCCs from patients with advanced disease.

- Nobre et al., (2021) Nat Cancer NIHMSID1675122
- Fane et al., (2021) Preprint – Research Square doi: 10.21203/rs.3.rs-61165/v1

2. Early dissemination and early DCC dormancy. Our lab has discovered that dormant breast cancer DCCs and metastasis can originate very early during cancer evolution, disseminating during premalignant stages and aided by innate immune cells. We identified a mechanism for early dissemination whereby Her2 aberrantly activates a program similar to mammary ductal branching that spawns early DCCs (eDCCs) capable of forming metastasis after a dormancy phase. We also revealed how the HER2 oncogene activates through CCL2 signaling the recruitment of tissue resident macrophages that help eDCCs to enter circulation. Targeting these macrophages early in cancer evolution reduced metastasis
late in cancer progression. Our focus is to understand how eDCCs found metastasis directly and/or through the preparation of eDCC-orchestrated pre-metastatic niches for later arriving DCCs to colonize target organs. We also aim to identify markers that might pinpoint early DCCs vs late DCCs and that may allow selectively targeting these cells.

- Nobre et al., (2021) Preprint – Research Square doi: 10.21203/rs.3.rs-145308/v1

3. **The balance between mitogenic and stress signaling as a determinant of dormancy.** We found that the ERK to p38 ratio was predictive of cancer cell dormancy or reactivation across different cancers, including HNSCC, breast and prostate. Our lab discovered that all cancer cells activate p38 upon dissemination but those that go on to metastasize from DCCs silence p38 signaling. We also found that p38 induced in dormant cells an unfolded protein response, upregulation of the chaperone BiP and activation of the ER kinase PERK. We also discovered that stress signaling activated by hypoxia in primary tumor microenvironments gives rise to a sub-population of dormant DCCs that evade therapy and may be the source of disease relapse and poor prognosis associated with hypoxia.


4. **Adhesion, stress signaling and autophagy.** We found that p38 is activated upon loss of integrin adhesion signaling leading to ERK1/2 inhibition and induction of the pro-apoptotic protein BimEL. This is key to induce anoikis, proper development of the mammary tree. We found that the HER2 oncogene inhibits p38 to accelerate cancer progression via anoikis resistance. We also discovered that proper adhesion signaling limits activation of the endoplasmic reticulum PERK, which limits mammary cancer initiation by blocking proliferation. We found that ErbB2 signaling is dependent on optimal activation of eIF2α signaling and that causing an imbalance in P-eIF2α levels killed ErbB2-overexpressing cells. We also showed that PERK-eIF2α-ATF4-CHOP activation in ECM-detached mammary epithelial cells induces autophagy and antioxidant responses for survival.

Our research is on the cellular and molecular mechanisms that maintain intestinal homeostasis, and perturbations that disrupt this causing intestinal and colonic disease - in particular, tumor development. This has involved development of mouse genetic models targeting intestinal cell maturation and lineage specific differentiation, and the impact of environmental alterations which in human populations are linked to altering probability of tumor development.

We demonstrated an orchestrated reprogramming of intestinal epithelial cells as they migrate from the progenitor cell compartment in the intestinal crypt and undergo maturation, eliminating cell cycling and promoting cell differentiation. We determined where key regulators of proliferation are active during this traverse of cells along the crypt-luminal axis, their oscillation along this axis, and effects of their disruption on mucosal homeostasis and tumor development.

The Profound Role of Diet in Both Genetically Initiated and Sporadic Tumorigenesis:

Colon cancer incidence in human populations is strongly linked to long term dietary patterns. For the mouse, this can be modeled using a purified rodent diet (NWD1). NWD1 incorporates changes of a number of key nutrients to mimic the level of intake of each to its level consumed in western populations with a much higher incidence of colorectal cancer. Feeding NWD1 accelerates and increases tumorigenesis in mouse genetic models of intestinal cancer, regardless of etiology, mechanism, or altered genetic drivers. For example, we collaborated with Winfried Edelmann on a mouse he engineered to be a faith-full genetic model for Hereditary Non-Polyposis Colon Cancer (HNPCC, or Lynch Syndrome). Feeding these mice NWD1 caused a major increase of tumor development in the large intestine, which is the site-specificity characteristic of individuals in human pedigrees who are carriers of inherited genetic mutations causal for Lynch Syndrome.

However, the vast majority (~80%) of human colon tumors are sporadic, developing without known inherited genetic factors, and arising after 5-6 decades of life with incidence largely determined by long term dietary patterns. Importantly, therefore, feeding NWD1 to wild-type C57Bl6 mice for 1-2 years caused sporadic small and large intestinal tumors with a lag, incidence, frequency and pathology similar to that of sporadic colon cancer in the human. This is the only mouse model of common sporadic tumors. Long before tumors developed, the mucosa, though seeming to function normally, exhibits distinct alterations at the cellular and molecular levels: there is macrophage infiltration into the mucosa, elevated serum cytokine levels, decreased secretory and increased absorptive cell lineage marker expression; and elevated Wnt signaling and ectopic Paneth cell marker expression throughout the small intestinal and colon mucosa.

These discoveries led to our current research on interaction of genetic and nutritional factors in the function of Lgr5hi intestinal stem cells. In summary, feeding the NWD1 (higher in fat, lower in vitamin D3, calcium, methyl donors and fiber), decreases the ability of Lgr5hi intestinal cells to function as stem cells in both intestinal homeostasis and in tumor development. An important role of lowered Vitamin D signaling in this was established in recapitulating the phenotype by targeted inactivation of the vitamin D receptor targeted to Lgr5hi cells. The dietary effect is linked to extensive transcriptional reprogramming of the Lgr5hi cells. For example, expression of the DNA mismatch repair pathway is elevated by lower vitamin D3 and/or calcium in the diet, paralleled by reduced accumulation of relevant somatic mutations detected by single Lgr5hi cell exome sequencing. In compensation, NWD1 also reprograms a second cell population - Bmi1+ cells - to function and persist as stem-like cells in mucosal homeostasis and tumor development. There is also a key role of compromised mitochondrial structure and function in inhibiting ability of Lgr5hi cells to function as stem cells, linked to down-regulation of Ppargc1a, a master regulator of Ppar signaling and mitochondrial biogenesis.

Key questions raised by the data are under investigation: a) What are the signals, and how are they transmitted, that recruit the Bmi1+ cells to function as stem cells? b) Is there a specific subpopulation of the heterogeneous Bmi1+ cell population that is mobilized, and what is its molecular phenotype (under investigation by single cell RNAseq). c) Since human diets vary considerably, how does this impact which and how different potential intestinal stem cell populations function in the population and can give rise to tumors? Further, are genetic, epigenetic and clinical phenotypes of colon tumors a function of this nutritional impact on stem cell populations? And what is the implication of nutritionally linked genetic and epigenetic alterations in relatively long-lived stem cells for effective approaches for prevention? d) Finally, experimentally induced damage to Lgr5hi cells can recruit multiple different intestinal cell populations to function as stem cells; our data demonstrate that this plasticity of function can also be marshalled by nutritional alterations. In this context, “adaptive radiation” – the rapid expansion of species and higher taxa to occupy new niches – is driven principally by ability of organisms to adapt to and utilize new sources of food. Therefore, an interesting hypothesis is that the plasticity of intestinal epithelial cells to function as stem cells may have arisen to provide flexibility to organisms to function in new ecosystems.
Selected Publications:


Regulation of Antibody Heavy Chain Gene Rearrangements and Expression by the 3' Regulatory Region

The immune system is our spacesuit for life in an environment containing enormous numbers of infectious agents. An essential part of the immune system is the B cell, which is the only cell type that produces antibodies. Antibody (Ig) genes are constructed via a series of DNA rearrangements. Occasionally, mistakes occur during antibody construction, which activate oncogenes and lead to cancers. My long term goal has been to understand the mechanisms that initiate and control antibody gene rearrangements within the 3 megabase heavy chain gene (IgH) locus.

Our experiments have focused on a complex 3' IgH regulatory region (3' RR) that lies immediately downstream of the antibody heavy chain gene cluster in mouse and humans. This region has recently been identified as a "super-enhancer" by Richard Young's laboratory, which activates the myc oncogene when it comes under its control as a result of chromosomal translocation. The ~50 kb 3' RR containing multiple regulatory elements, a 1 kb intronic enhancer, and two small elements in the diversity DH gene region are the only currently known long-range regulatory regions for the IgH locus. We have identified an extension of the 3' RR (hs5, 6, 7, 8), which like the other 3' enhancers, has binding sites for Pax5, a transcription factor essential for B cell development. In addition, the 3' RR extension contains insulator activity, i.e. prevention of communication between an enhancer and its target promoter. This is associated with multiple binding sites for CTCF, a protein identified in all mammalian insulators. Chromatin analysis shows that the 3' RR extension is likely to be active throughout B cell development while other 3' RR segments become progressively and stage-specific active.

Murine germline IgH locus, with variable (VH), diversity (DH), joining (JH) and constant region genes (\(\mu\), \(\delta\), \(\gamma\), \(\alpha\), \(\epsilon\), \(\lambda\)). Regulatory elements, E\(\mu\) and the 3' regulatory region (3'RR), are depicted. Two CTCF sites in the DH segment are important for VDJ joining.

The 3' RR has been shown to be critical both for class switch recombination that affects expression of virtually all antibody classes and for somatic hypermutation of the heavy chain variable region. In addition, the 3' RR regulates high levels of expression of antibodies in fully differentiated plasma cells. A major goal of our laboratory has been to understand the mechanisms by which the 3' RR functions normally in both mouse and human, both by acting on the IgH locus and by insulating the locus from its non-Igh neighboring genes. CTCF binding sites are anticipated to play a major role in these activities by promoting interactions between distal DNA sequences through loop formation.

We have been analyzing the extent of B cell-specific regulation of the IgH locus, by studying DNA demethylation, histone modifications of and the binding of CTCF and Pax5, and other factors to 3' RR sequences during B cell development and class switching. Furthermore, we have examined the interaction of the 3' RR with target sequences within the IgH locus, as assessed by the
chromosome capture conformation technique (3C). Together, these approaches have been designed to understand how the 3' RR facilitates molecular acrobatics necessary for the immunoglobulin heavy chain locus without their intrusion on downstream non-IgH genes, or resulting in chromosomal translocations involved in malignancies.

Publications:


**Key Words:** Red Blood Cells, Sickle Cell Disease, Epigenetic, gene regulation, stem cells, reprogramming.

**CULTURE RED BLOOD CELLS:**
We have developed a method to produce genetically engineered human red blood cells by differentiation of human induced-Pluripotent Stem Cells. We have several ongoing projects aiming at producing large amount of genetically homogenous, genetically modified red blood cells that will be used as reagent red blood cells and for transfusion of allo-immunized sickle cell disease patients, and as carrier for replacement therapy of a variety of diseases including TTP, hemophilia A and several coagulation blood disorders.

**GENE THERAPY:**
We are studying the biology of hematopoietic stem cells in the context of sickle cell disease and are developing CRSPR-based methods to genetically modify these cells for gene therapy using a safe harbor approach.

**HUMAN EMBRYONIC STEM CELLS, EPIGENETICS AND REPROGRAMMING:**
Epigenetic is the study of mitotically or meiotically heritable changes in gene function not associated with changes in DNA sequence. Epigenetic regulations are mediated by changes in chromatin structure that alter access of transcription factors to their cognate binding sites, and therefore, expression levels of genes and transgenes. Understanding these regulations is critical for gene therapy, cancer therapy and generally to gain a greater ability to modify mammalian genomes. We have several ongoing basic science projects to study these questions in human iPSCs and hematopoietic cells.

**FOR MORE DETAILS:** https://www.einstein.yu.edu/faculty/4981/eric-bouhassira/

**Selected References:**


Genomic Instability and Cancer in Murine Models

The maintenance of genomic integrity in all organisms requires multiple DNA repair pathways that are involved in the processes of DNA replication, repair and recombination. Perturbations in these pathways can lead to increased mutation rates or chromosomal rearrangements that ultimately result in cancer. MMR is one of the repair systems that mammalian cells employ to maintain the integrity of its genetic information by correcting mutations that occur during erroneous replication. Mutations in MMR genes are linked to one of the most prevalent human cancer syndromes, Lynch syndrome and a significant number of sporadic colorectal cancers. At the molecular level tumors that develop in these patients display increased genomic mutation rates as indicated by increased instability at microsatellite repeat sequences (termed microsatellite instability, MSI). MMR in eukaryotes is complex and involves several homologs of the bacterial MutS and MutL proteins. In mammals, the initiation of the repair process requires two complexes formed by three different MutS homologs (MSH): A complex between MSH2-MSH6 for the recognition of single base mismatches and a complex between MSH2-MSH3 for the recognition of insertion/deletions. The repair reaction also requires a complex between the two MutL homologs MLH1 and PMS2 that interacts with the MSH complexes to activate subsequent repair events which include the excision of the mismatch carrying DNA strand and its re-synthesis. In addition to correcting DNA mismatches, the MMR system mediates an apoptotic response to DNA damage and both of these functions are thought to be important for genome maintenance and tumor suppression. We have generated gene targeted mouse lines with inactivating mutations in all the different MutS and MutL homologs, and also in genes that function in the later MMR steps to study their roles in genome maintenance and tumor suppression. In addition, we have generated knock-in mouse lines with missense mutations and conditional knockout mouse lines that inactivate specific MMR functions and/or model mutations found in humans. Our studies indicate that specific MMR functions play distinct roles in maintaining genome stability and that defects in these functions have important consequences for tumorigenesis. These studies have also revealed that MMR proteins play essential roles in class switch recombination and somatic hypermutation during antibody maturation and the control of meiotic recombination in mammals. We are currently studying the functions of MMR in intestinal stem cells (ISCs) and cancer stem cells (CSCs) in preclinical mouse models and how loss of MMR in stem cells affects tumorigenesis and the response of tumors to anticancer treatment.

Selected References:


Our laboratory has three areas of interest:
* denotes first authorship by a student in the Frenette lab.

A) We are interested in the biology of hematopoietic stem cells (HSCs) with a focus on microenvironment cues that promote their survival, differentiation and self-renewal. We have identified novel niche constituents and novel regulatory mechanisms using genetically engineered mice and whole-mount imaging of the bone marrow.

Key primary references:


B) We have ongoing projects on the mechanisms of vaso-occlusion in sickle cell disease. We have identified aged neutrophils as a key promoter of vaso-occlusion by interacting with circulating sickle erythrocytes, and shown that neutrophil aging in the circulation was driven by the microbiota.

Key primary references:


C) Based on our finding of an important role of neural signals in regulating hematopoiesis, we are investigating the role of peripheral nerves in hematologic malignancies and prostate cancer. In particular, our studies show that neural signals regulate the metabolism of endothelial cells, thereby regulating angiogenesis.

Key references:


BIOCHEMISTRY AND GENETICS OF CHROMATIN TRANSITIONS IN DROSOPHILA

Hundreds of millions of base pairs of nuclear DNA are packed into chromosomes. Chromatin, the nucleoprotein filament of a chromosome, has many organization levels. It is the natural state of DNA in the nucleus and the native substrate for DNA-directed reactions, such as DNA replication, recombination, repair and transcription. The assembly of DNA into chromatin and dynamic conversion between its different forms are critical steps in the maintenance and regulation of the eukaryotic genome. The goal of our research is to understand how chromosomes are assembled and how this process regulates the structure and activity of euchromatic chromosomes. The crucial first step in this direction is a systematic study of factors that mediate this process. To this end, we use biochemical approaches to analyze mechanisms of chromatin assembly by histone chaperones and ATP-driven enzymes. We also dissect their function in vivo by methods of Drosophila genetics. Thus, we are trying to uncover the network of chromatin assembly factors and to elucidate their roles in hierarchical organization of the chromosome.

1. Molecular mechanisms of nucleosome assembly

ACF (ATP-utilizing chromatin assembly factor) was identified on the basis of its ability to facilitate reconstitution of chromatin in vitro. It consists of two subunits, a SNF2-like ATPase ISWI and a polypeptide termed Acf1. In conjunction with a core histone chaperone NAP-1, ACF mediates deposition of histones onto DNA and forms arrays of regularly spaced nucleosomes. We study ACF as a prototype factor to elucidate molecular events that take place during ATP-dependent formation of nucleosomes. During assembly, ACF commits to the DNA template and forms nucleosomes as a processive, ATP-driven, DNA-translocating motor. Multiple conserved domains of Acf1 and ISWI are required for this activity.

2. Biological functions of chromatin assembly factors

ACF is the major ATP-dependent assembly factor in Drosophila. To expose its biological functions, we produced fly mutants that do not express ACF. ACF-deficient animals have multiple defects of chromatin organization. However, ACF is not essential for viability due to the presence of redundant ACF-like factors. We discovered novel ISWI-containing complexes ToRC (comprising Tou, ISWI and CtBP) and RSF (Rsf1 and ISWI) that can functionally substitute ACF in vivo. Our genetic and cytological analyses implicate the network of ATP-dependent, ISWI-containing chromatin assembly factors in diverse, partially redundant pathways of regulation of chromatin structure and activity.

SNF2-like protein CHD1 is another ATP-dependent nucleosome assembly factor. We disrupted Chd1 in flies and discovered that CHD1 is required for replication-independent deposition of histones into chromatin in vivo. Specifically, CHD1 is essential during early embryonic development for deposition of replacement histone H3.3 into paternal chromatin.

3. Higher-order chromatin forms

To reconstitute higher-order chromatin structures, we supplement the in vitro assembly system with modified core histones, histone variants, linker histone (H1) and heterochromatin proteins, such as Drosophila HP1a. Chromatin vectors can turn into useful tools in research and therapy. These studies will also eventually lead to the discovery of techniques to reconstitute functional metazoan chromosomes.

In collaboration with A. Skoultchi, we began to examine flies in which H1 is depleted by RNAi or genetic approaches. We discovered that H1 is the major component of heterochromatin and is required to establish its biochemical identity and functional properties. For instance, H1 recruits HMT Su(var)3-9, which mediates methylation of lysine 9 of histone H3 (H3K9), a signature heterochromatin-specific epigenetic mark. We have also demonstrated that H1 is essential for the faithful regulation of DNA endoreplication timing in Drosophila larval cells. We are now extending these studies to normal, mitotically dividing cells.

A prevalent view of heterochromatic silencing is that its physical compaction results in steric exclusion of regulatory proteins, such as RNA polymerases. In collaboration with G. Karpen (LBNL), we have recently shown that the formation of heterochromatin domains is also mediated by liquid-liquid phase separation that gives rise to a non-membrane-bound nuclear compartment. We demonstrated that HP1a and H1 undergo...
demixing in vitro and nucleate into foci that display liquid properties during heterochromatin domain formation in early Drosophila embryos. We propose that biophysical properties associated with phase-separated systems are critical to understanding the behavior of heterochromatin and, potentially, other chromatin forms that regulate essential nuclear functions.

4. Sperm chromatin assembly and remodeling

In sperm, DNA is compacted with cysteine-rich protamines and protamine-like sperm nuclear basic proteins (SNBPs) to form enzymatically static sperm “chromatin”. We have begun to analyze protein factors that mediate SNP deposition during spermatogenesis and their removal from DNA after fertilization. It turns out that sperm chromatin assembly and remodeling is mediated by a group of factors that are similar to core histone chaperones.

Upon deposition on sperm DNA, protamines/SNBP are extensively crosslinked via interchain disulfide bonds. After fertilization, the egg has to reverse the crosslinks for efficient eviction of SNBPs. This nuclear reaction is mediated by specific thioredoxin (TRX) and thioredoxin reductase (TRXR) molecules. Thus, we are investigating biological roles of the evolutionary conserved thioredoxin system in sperm chromatin metabolism and female fertility. A number of chemical compounds are known to specifically inhibit the function of TRX and TRXR proteins. We are studying their ability to suppress fertilization in the egg in vivo and testing their utility as novel, non-hormone agents for female contraception.

Selected publications
MacroH2As, histone variants with diverse roles in gene expression and DNA damage responses

- The macroH2A-type histone variants (which include macroH2A1.1, macroH2A1.2 and macroH2A2) have roles in tumor suppression, cellular senescence, activation and repression of transcription, promotion of DNA repair and suppression of the reprogramming of differentiated cells into stem cells. MacroH2As are typified by a histone H2A-like region fused by a flexible linker to a C-terminal macrodomain, a ligand-binding domains whose functions is modulated by binding to poly(ADP-ribose) produced by a family of poly(ADP-ribose) polymerases. MacroH2A1 regulates the expression of genes found within its large chromatin domains which can span hundreds of kilobases. Through changes in its expression and/or alterations in its genomic localization, disruption of macroH2A1’s tumor suppressive functions are common in cancer; alterations of macroH2A transcription and splicing occur in a variety of cancers including those of lung, breast, colon, ovaries, endometrium, bladder, testicles, and melanocytes. Consistently, macroH2A1 loss in primary cells is sufficient to trigger an oncogenic gene expression profile. We are interested in many aspects of macroH2A biology. 1) How are macroH2As targeted to specific regions of the genome? 2) How does macroH2A1.1 in collaboration with PARPs regulate gene expression? 3) How does macroH2A1 regulate chromatin accessibility at enhancers? 4) How does macroH2A participate in DNA repair? 5) What regulates macroH2A1’s alternative splicing?

Chromatin dynamics during oncogene-induced senescence and cancer

- Oncogene-induced senescence (OIS) is an important tumor suppressive mechanism whereby a cell harboring an oncogenic mutation enters a stable proliferative arrest. At the same time the senescent cell secretes a host of inflammatory cytokines, chemokines and metalloprotease called the senescence-associated secretory phenotype (SASP), which serves to recruit immune cells to clear the senescent cells from tissues. The histone variant macroH2A1 plays a critical role in the transcriptional regulation of SASP genes during senescence. We are currently studying the mechanism by which macroH2A regulates the SASP response. We hypothesize that changes in macroH2A1 expression, seen in many cancers, allows these cells to bypass senescence and proceed on the pathway towards transformation.

Interplay between transcriptional elongation rates and alternative splicing

- Alternative splicing is a crucial aspect of gene expression, allowing a gene to yield functionally distinct products, the abundance of which are regulated by cellular cues. Splicing dysregulation is central to several cancers and developmental diseases. Alternative splicing can be regulated through the recruitment of splicing factors which promote or repress distinct splicing events. Splicing largely occurs co-transcriptionally, and so, splicing outcomes are also affected by aspects of the transcription process and chromatin environment. The local elongation rate of RNA polymerase II is one aspect of transcription with important consequences on splicing outcomes. A barrier to progress in the field has been the lack of a high-throughput assay to measure splicing rates in mammalian cells. To address this, we have developed SKaTER-seq (Splicing Kinetics and Transcript Elongation Rates through sequencing). With this assay, we are exploring a myriad of factors that regulate splicing, including elongation rate, gene architecture, binding sites for RNA binding factors, chromatin structure and histone modifications. With this powerful approach we will determine the underlying causes of splicing alterations in disease.

Selected References:


The PI3 kinase Signaling Pathway in Adult Blood Development and Leukemia

My lab studies the signal transduction pathways that affect the early fate decisions of adult hematopoietic stem cells (HSCs) as they progress from an undifferentiated multipotent state to the generation of differentiated blood cells. When these early fate decisions go awry, this can lead to the formation of leukemic stem cells, which can initiate leukemia and contribute to relapse after treatment.

Roles of the PI3 kinase isoforms in adult blood development

PI3 kinase (PI3K) is a lipid kinase that is important for the regulation of metabolism, the cell cycle, apoptosis, and protein synthesis. In hematopoietic cells, there are four isoforms of the catalytic subunit of PI3K, each encoded by a separate gene. Emerging evidence suggests that these isoforms have unique functions in normal and cancer cells, but may substitute for each other in some contexts. We have generated a series of mouse knockout models that allow us to study the roles of each of these isoforms individually in adult hematopoiesis. For example, we have found that the p110alpha isoform is most important for red cell development, but is not required in normal blood stem cells. We have now also generated compound knockout mice to determine the redundant roles of the PI3K isoforms in blood development. We are studying how deletion of PI3K impacts normal HSC function, including self-renewal, proliferation after infection or chemotherapy treatment, and differentiation along different blood lineages.

Roles of the PI3 kinase isoforms in leukemia

Acute myeloid leukemia (AML) is a genetically diverse disease, but activation of the PI3K pathway has been reported in up to 80% of cases. A subset of AML cell lines and AML patient samples respond to PI3K pathway inhibitors, but it is unclear how patients should be selected for potential response to these inhibitors. We found that RAS-mutated myeloid leukemias are particularly dependent on the p110alpha isoform of PI3K, and that pharmacologic inhibition of p110alpha can be used to treat both RAS-mutated cell lines and RAS-mutated leukemia in mice. We are now using several different mouse models of AML to examine the roles of individual PI3K isoforms in leukemic stem cells, which is the cell population that has been implicated in relapse. We are also studying the roles of PI3K in the normal and leukemic bone marrow microenvironment.

Selected Publications


My lab is interested in two interlocking areas of stem cell biology and cancer biology: the molecular pathways that regulate the normal stem-cell fate in the mammary gland, and the role of stem cell fate/pathway dysregulation in breast cancer pathogenesis.

**Role of mammary stem cells in cancer initiation**
We have developed sensitive and specific in vitro and in vivo mammary stem cell assays. Using these assays, we have identified novel unipotent stem cells that are responsible for the development of distinct mammary epithelial cell lineages. We are investigating whether these unipotent stem cells are the cell-of-origin for different breast cancer subtypes. In addition, we are elucidating the key cell fate determinants of these distinct stem cells, and investigating oncogenic mechanisms causing the dysregulation of stem cell fates during tumorigenesis.

**Function of stem-cell pathways in breast cancer progression and metastasis**
Emerging evidence suggests that normal stem-cell pathways often get activated aberrantly in cancers and contribute to aggressive cancer behaviors. Identification of key normal stem cell regulators provides us a framework to understand how breast cancer stem cells are regulated. We are particularly interested in understanding the role of stem-cell factors in regulating metastatic colonization, a rate-limiting step of the metastatic cascade that involves cancer stem cells. In addition, we are interested in how cancer stem cells are regulated by the tumor microenvironment.

**Selected Publications**


Keisuke Ito, M.D., Ph.D.
Associate Professor

Keisuke Ito, M.D., Ph.D.
Associate Professor

key words: Hematopoietic stem cell, Leukemia, Myelodysplastic syndrome

The central research goal of the Ito Lab is the expansion of our understanding of the regulatory pathways controlling the equilibrium of stem cells, with a special focus on the development of novel therapeutics for hematopoietic disorders. At the core of our work is the process of stem cell division, and the resulting balance between self-renewal or differentiation, which directly impacts tissue homeostasis. We are also devoting increased attention to targeting cellular metabolism as a therapeutic strategy, and have cut a path along the leading edge of research into the role of epigenetic-microRNA crosstalk, including physiologically relevant Ten-eleven translocation, in the pathogenesis of myelodysplastic syndrome. We believe our expertise in stem cell biology, hematology, and the bone marrow microenvironment, combined with our development of single-cell approaches and live imaging to tracking stem cell fate in animal models, will together facilitate a major contribution to the improvement of transplantation efficiency and the development of new therapies and treatments, and potentially even cures, for hematologic pathology.

Selected Original research and Theoretical treatises;


Selected Invited publications;

Molecular Mechanisms of Virus Entry and Exit.

For more information, please see our lab homepage: https://sites.google.com/site/kielianlab/

All enveloped viruses use the essential steps of membrane fusion to enter a host cell, and membrane budding to exit. Molecular information on the entry and exit processes is critical to understanding the lifecycle of enveloped viruses and how they exploit the host cell machinery, and as a key model for cellular membrane fusion and budding reactions.

Our research focuses on the molecular mechanisms of virus entry and exit using alphaviruses and the closely related virus Rubella virus, and flaviviruses such as dengue virus. The flaviviruses and alphaviruses include many important human pathogens such as dengue, Zika, and Chikungunya viruses, which cause millions of human infections each year. There are no vaccines or antiviral therapies for most of these viruses, and new strategies are urgently needed.

Alphaviruses, Rubella virus and flaviviruses enter cells by endocytic uptake and then fuse their membrane with the endosome membrane in a reaction triggered by the low pH of the endocytic vesicle. The membrane fusion proteins of these viruses are structurally related proteins and refold during fusion to a homotrimer conformation that mediates virus fusion and infection. Recent studies have also shown that structurally similar proteins are expressed in plants and in many animals, where they mediate cell-cell fusion of gametes and during development.

Many important questions on the molecular mechanism of membrane fusion remain for both viruses and cells. Little is known about the mechanism and structural features of fusion protein insertion into the target-membrane. We are also investigating the pH-dependent control mechanisms for the Rubella virus fusion reaction.

During alphavirus and flavivirus biogenesis, a companion protein forms a closely-associated dimer with the fusion protein, and protects it from low pH and premature fusion during exocytic transport. This companion protein must then dissociate to permit virus fusion. The pH protection mechanisms for many other viruses are unknown, and we are using Rubella virus as a system to define novel mechanisms of pH protection.

Alphaviruses exit by budding through the plasma membrane of the infected host cell. Little is known about alphavirus assembly and budding, although it is clear that these processes are highly regulated to produce organized virus particles of high specific infectivity. How does this happen and what are the roles of cellular and viral factors? We seek to determine how the internal viral RNA-capsid core is assembled, how the virus excludes host RNAs, and how nucleocapsid assembly can be inhibited by small molecules. We are using a novel capsid protein retrieval strategy to identify and characterize host factors involved in alphavirus nucleocapsid assembly. We have developed fluorescently tagged alphaviruses to follow virus assembly and budding in real time in infected cells. We are investigating how alphaviruses spread from cell to cell, a process that protects the virus from antibody neutralization. The cell plasma membrane and cytoskeletal network are dramatically remodeled during budding and we are defining the mechanisms and signaling pathways that mediate remodeling.
Our lab uses a wide variety of approaches including molecular biology, virus genetics, protein biochemistry, live cell imaging, cell biology, and structural biology. Potential research projects include: investigation of specific molecules involved in cell-to-cell virus transmission, use of fluorescently tagged viruses to follow steps in virus assembly and budding, characterization of the role of cellular factors in virus assembly and exit, use of virus mutants to characterize specific steps in fusion and pH protection.

Selected references:
Fundamental mechanisms of cell death; molecular connections among death programs; development of small molecule drugs to manipulate cell death in human disease

Cell Death: Fundamental Mechanisms and Roles in Human Disease

The most basic decision that any cell makes is to grow, differentiate, or die. Our laboratory studies fundamental mechanisms of cell death and the roles of cell death in normal biology and human disease.

Fundamental research Twelve cell death programs have been recognized to date. Little is understood regarding how they coordinate and integrate to produce a unified cell death response. We have focused on two of these cell death programs, mitochondrial-mediated apoptosis and mitochondrial-mediated necrosis. The defining molecular events that activate each of these programs take place within microns of each other at the outer and inner mitochondrial membranes respectively. We are attempting to delineate how these cell death programs interconnect mechanistically and functionally. We have identified proteins that unite apoptosis and necrosis signaling, and therefore may serve as "decision points" between these death programs. One is the cell death inhibitor ARC that antagonizes multiple apoptosis and necrosis pathways (Molecular Cell, 2004; PNAS, 2007; JBC, 2007; Cell Death Differ, 2014). Another is the BCL-2 protein BAX, which has been long recognized for its role in apoptosis and which we have discovered is critical for mitochondrial-mediated necrosis through distinct mechanisms (PNAS, 2012). Also related to mitochondrial cell death signaling, other work in the lab is focused on understanding how alterations in the conformations of mitofusins regulate mitochondrial-ER interactions to impact metabolism and cell death (based on Nature, 2016; https://www.biorxiv.org/content/early/2018/04/17/301713; Science, 2018); and on functions of the F1-Fo mitochondrial ATP synthase.

Translational research. While we have studied roles of cell death in cancer (Cell Death Differ, 2005; JBC, 2010; Cancer Res, 2011; PLoS One, 2015), diabetes (Diabetes, 2013; Sci Rep, 2017; Developmental Cell, 2021), and pulmonary hypertension (Circulation, 2011), our most important translational accomplishments have focused on cell death in heart disease - specifically in the most common and lethal cardiac syndromes - myocardial infarction (heart attack) and heart failure. Our lab was one of the founders of the cardiac cell death field and has played a major role in its development (Physiol Rev, 2019), including the first demonstrations that regulated forms of cell death play central roles in the pathogenesis of myocardial infarction (J Mol Cell Cardiol, 2000) and heart failure (J Clin Invest, 2003). Currently, our translational work is focused on the chemical biology of cell death and, specifically, the development of small molecule drugs to inhibit apoptosis and necrosis. We have employed both unbiased phenotypic screening of large chemical libraries (Probe Reports from the NIH Molecular Libraries Program, 2013) and target-based approaches. The latter efforts have focused on BAX (Nat Chem Biol, 2019) because of its dual regulation of apoptosis and necrosis. We are studying BAX inhibitors in several clinically relevant contexts including doxorubicin-induced cardiomyopathy (Nat Cancer, 2020).

Our experimental approaches include biochemistry, molecular and cellular biology, the creation of genetic mouse models of disease; and, in collaboration with the Gavathiotis lab, chemical and structural approaches to small molecule drug design.

The laboratory currently consists of 12 individuals including one Instructor, one Research Scientist, 3 postdoctoral fellows, 6 graduate students (5 Ph.D. program; 1 M.D./Ph.D. program (NIH MSTP)), and one technician. An important facet of my work is training and mentorship. I have been thesis research advisor to 25 individuals who have received or are pursuing the Ph.D. degree, ~50 postdoctoral research fellows, and ~10 clinical fellows. A significant proportion of my trainees have gone on to academic faculty positions as independent investigators and include a number of individuals from groups under-represented
in science.

Selected Publications

Selected publications last 5 years and older publications as cited above:


Gene regulatory mechanisms that mediate lung cancer evolution

Lung adenocarcinoma (LUAD) is the most common subtype of non-small cell lung cancer and remains a leading cause of cancer-related death. Next-generation sequencing studies have identified a number of recurrent genetic drivers (such as mutations in \textit{KRAS} and \textit{TP53}); however, this somatic variation does not fully explain key features of cancer evolution including progression toward metastasis and intratumoral heterogeneity. Our group aims to understand how non-genetic mechanisms contribute to lung cancer initiation and progression through the lens of chromatin biology.

In genetically engineered mouse modeling studies, cell-type specific transformation of alveolar type II (AT2) cells with \textit{Kras}^{G12D} and loss of \textit{Tp53} lead to a natural cancer evolution process that recapitulates phenotypic hallmarks of human disease. Using this model, our recent work employed single-cell epigenomics to study dysregulation of chromatin state across lung cancer evolution. We identified a continuum of heterogeneous chromatin states with reproducible epigenomic features across individual lung tumors, suggesting conserved routes of cancer progression. Using both functional and computational approaches, we annotated transcription factor (TF) regulators and downstream gene programs associated with these diverse chromatin profiles. These discernable gene regulatory modules were linked to certain aspects of cancer progression including loss of cellular identity, inflammation, and metastasis. Our work also identified the emergence of a pre-metastatic gene regulatory program that arises in a rare cell population in primary tumors and primes cells for metastatic seeding. We identified \textit{RUNX2} as an important regulator of this cell state and are developing new models to study the role of \textit{RUNX} TF biology in lung cancer progression.

Cellular identity is dictated by chromatin structure, which is maintained by a complex network of \textit{cis-} (e.g., enhancer, promoters, and insulators), and \textit{trans-} (e.g., chromatin modifiers, TFs, and adaptors) factors. Our previous work found that early stages of LUAD progression are associated with the loss of AT2 identity and the acquisition of epigenomic states similar to developmentally related cell types. We seek to further understand how lung cancer initiation ultimately leads to decoupling from an AT2 cell identity and increased epigenomic plasticity. In addition, we are interested in investigating how disruption of the tumor microenvironment influences LUAD cell state. To address these questions, we pair lung cancer modeling with epigenomic technologies (including single-cell ATAC-sequencing, single-cell multiomic analysis, lineage tracing, and spatial approaches) to dissect mechanisms important for the maintenance and persistence of dysregulated cell states in lung cancer. We flexibly utilize genetically engineered mouse models, alveolar human and murine organoids, and cell-based assays to perform functional studies. We continue to expand our technological and modeling toolkit to study early and late stages of lung cancer progression with the overarching goal of better understanding and targeting lung cancer progression.

**Research directions in the lab:**
1. Perturbation of candidate chromatin regulators associated with aberrant cell states in lung cancer
2. Interrogation of mechanisms that drive epigenomic plasticity and cell identity shifts in LUAD
3. Investigation of the role of the tumor microenvironment and loss of tissue homeostasis in shaping cell state in lung cancer cells
4. Utilization of varied single-cell epigenomic technologies to decipher gene regulatory networks in normal lung and lung cancer cells
Selected Publications:

*Co-first; *Co-corresponding authors

*Co-corresponding authors


*Co-corresponding authors


*Co-first authors
Genetics of Blood Cancer Predisposition

Our laboratory is interested in understanding how inherited genetic variants contribute to hematologic malignancy. Myeloid malignancies are caused by acquired somatic mutations or chromosomal aberrations that result in dysregulation of cancer driver genes. These somatic driver mutations occur more frequently on a background of particular inherited germline variants. We are leveraging genome-wide association studies (GWAS) to prioritize germline genetic variants and identify mechanisms predisposing to myeloid malignancies and pre-leukemic conditions such as clonal hematopoiesis. Using CRISPR/Cas9 approaches and high-throughput screens in human hematopoietic stem and progenitor cells (HSPCs) and engineered mouse models, we are studying the impact of such genetic risk variants on hematopoiesis.

Role of hematopoietic stem cell (HSC) expansion in myeloid malignancy risk

Our recent work has uncovered a previously unappreciated mechanism for inherited risk of myeloid malignancies involving modulation of HSC function and self-renewal (Bao et al., Nature 2020; Bick et al., Nature 2020). We showed that non-coding germline variants identified from GWAS on myeloproliferative neoplasms and clonal hematopoiesis, influenced hematopoietic enhancers resulting in expansion of phenotypic human HSCs. We are currently developing murine models carrying germline deletions/point mutations of syntenic murine enhancers. Using these models, we will examine how modulating HSC pool size may predispose to myeloid malignancies.

Interactions between germline variants and somatic mutations underlying clonal hematopoiesis.

Clonal hematopoiesis is an age-related premalignant condition characterized by expansion of blood cell clones carrying somatic mutations. GWAS have identified several genetic loci that predispose to clonal hematopoiesis, but underlying mechanisms remain unknown. In this project, we are examining functional interactions between GWAS nominated germline variants and common somatic mutations observed in clonal hematopoiesis. We are engineering human HSPCs using CRISPR/Cas9 to model both germline and somatic variants and examine clonal expansion using in vitro and in vivo assays.

High throughput approaches to systematically connect non-coding GWAS variants to genes regulating HSCs.

The high prevalence of non-coding genetic variants presents a major challenge to GWAS functional validation studies. We are developing two high-throughput approaches in human HSPCs that are independent and complementary to systematically examine non-coding variants impacting blood cells. 1) A lentiviral massively parallel reporter assay (LentiMPRA) that uses DNA barcodes to quantify reporter activity of non-coding regions at high throughput in human HSPCs (Ulrich et al., Cell 2016). 2) A pooled CRISPR/Cas9 screen to endogenously disrupt/delete GWAS nominated putative enhancers in human HSPCs. We will apply these approaches to systematically identify the affected enhancers and genes at GWAS loci associated with clonal hematopoiesis and test effects of genetic variants on HSC function.

Selected Publications


DOI: 10.1038/s41586-020-2786-7


DOI: 10.1038/s41586-020-2819-2


DOI: 10.1016/j.tig.2020.05.006


DOI: 10.1007/s10875-020-00778-7


DOI: 10.7554/eLife.44080.


DOI: 10.1016/j.celrep.2019.05.046.


DOI: 10.1073/pnas.1619052114.


DOI: 10.1016/j.cell.2016.04.048.


DOI: 10.1111/bjh.13938.


#Corresponding author.


Google Scholar Link

https://scholar.google.com/citations?user=6hnA6y4AAAAJ&hl=en
RNA Processing – Mechanisms and Disease

Intron removal, a defining feature of eukarya, is catalyzed by the spliceosome, a 50-60S complex composed of five snRNAs and >100 proteins. Our laboratory investigates spliceosome assembly and catalysis and the mechanisms by which spliceosomal mutations cause disease. We have developed orthogonal (or ‘designer’) spliceosomes to facilitate investigation of core RNA–RNA interactions, and we have identified new spliceosomal snRNAs in the human genome and novel snRNA mutations prevalent in some cancers.

Do variant snRNAs make variant spliceosomes? One ‘black hole’ in RNA biology is the identification of 50-300nt RNAs, missing in modern-day sequencing datasets. Vertebrate genome sequences reveal hundreds of snRNA gene loci; with few exceptions, only the most abundant canonical snRNAs have been investigated. We are sequencing the snRNA transcriptome, asking which variants are expressed in different tissues and disrupting variant snRNA loci. We find that variant-snRNA expression changes greatly during development. How does this impact spliceosome function?

Orthogonal systems for in vivo investigation of catalytic center interactions Branch site (BS) base pairing with U2 snRNA is essential for spliceosome assembly and first-step catalysis. Investigation of this duplex was previously limited by the deleterious nature of mutations that disrupt it. We developed an orthogonal system wherein a second-copy U2 with grossly substituted BS-U2 base-pairs mediates splicing of a cognate reporter gene. This orthogonal BS-U2 pair produces a non-essential second spliceosome that allows in vivo characterization of the BS-U2 duplex and its interaction with the spliceosome core. These properties allowed us to demonstrate that the BS-U2 duplex exists at the time of first-step catalysis.

What is the second-step catalytic core? We used our orthogonal systems to elucidate a 3'SS binding site within the second-step core, demonstrating that the first-step branch structure becomes partially unpaired from U2-GUAGUA, allowing the 3'SS to bind on U2 snRNA.

Splicing and disease U2 snRNP protein SF3b1 and other U2 proteins highly mutated in myelodysplastic syndromes and other cancers. However, there has been no information regarding U2 snRNA mutations. Our analysis of U2 variant loci allowed us to interrogate U2 RNA mutations in more than 1000 WGS from the TCGA. Our preliminary data identify mutations in both U2-1 and U2-2 snRNAs, within the BSL, recurrent in bladder cancer; and we have identified the same mutations in a collection of bladder cancer organoid lines (Lee et al., Cell 2018). The BSL contributes to recognition of the branch site as well as 3'SS. Our genetic screens in yeast for improved splicing of non-consensus 3'SS yield the same BSL.
mutation as found in bladder cancer. We are investigating the molecular mechanisms of this dysregulation, using both yeast and mammalian systems.

**Recent Publications** (selected)


Highlighted as a “Breakthrough Paper”: [http://www.narbreakthrough.com/2020/05/13/prp5](http://www.narbreakthrough.com/2020/05/13/prp5).


Our laboratory is studying how antibody-forming cells respond to antigen by undergoing somatic hypermutation and class switch recombination so that they can produce higher affinity antibodies with more useful effector functions. The molecular and biochemical mechanisms of antibody variable region hypermutation and class switch recombination is being studied in mice that have mutations in various repair proteins in collaboration with Dr. Winfried Edelmann. In order to examine detailed molecular mechanisms, we are also studying how mutation is targeted to antibody genes and some oncogenes in human Burkitt’s lymphoma cell lines which are undergoing variable region mutation in culture. These cell lines are being used to study the role of activation induced deaminase (AID), mismatch repair and error prone polymerases in the variable region hypermutation and isotype switching. The analysis of these events also involves the examination of how changes in the DNA sequence of antibody genes, chromatin structure and transcriptional elongation lead to the targeting and regulation of AID. In collaboration with Dr. Thomas MacCarthy at Stony Brook, the role and evolution of the antibody V region sequence is also analyzed computationally using data bases of the human antibody response. The highly mutagenic processes required to generate antibody diversity also leads to B cell lymphomas and we are trying to understand how AID contributes to human Chronic Lymphocytic Leukemia in collaboration with Dr. Nicholas Choirazzi from the Feinstein Institute at the Northwell Health Center.

We are also the Hybridoma Facility that helps investigators throughout the institution to make their own monoclonal antibodies.

Selected References:


7) A source of the single stranded DNA substrate for activation induced deaminase during somatic hypermutation. Xiaohua Wang, Manxia Fan, Susan Kalis, Lirong Wei, Matthew D. Scharff Nat. Communications 2014 Jun 13;5:4137. doi: 10.1038/ncomms5137. PMID: 24923561 PMCID: PMC4154566


Our laboratory is a part of the Einstein Center for Human Embryonic Stem Cell Research and the Cancer Center.

**Molecular Analysis of DNA Replication and Repair in Alzheimer's Disease and at Cancer-Associated Sites in the Human Genome**

A major interest of our laboratory is how the DNA replication program in mammalian cells is organized and regulated. We are currently focused on understanding the role of genomic instability at DNA loci that have been implicated in Alzheimer's disease (AD) and prostate cancer. AD is the major cause of dementia affecting more than 40% of elderly individuals and prostate cancer is the second leading cause of death in American men.

We also study human chromosomal fragile sites in cancer (including mechanisms of chromosomal translocations), aging disorders related to trinucleotide repeat expansion, telomere replication and reprogramming of DNA replication in human embryonic stem (ES) cells.

After many years of study there is still no cure for AD. We plan to take a novel approach to study the impact of DNA replication of certain regions of the genome that have been implicated in causing AD. This would lead to rational strategies that target the replication mechanisms and to therapies that could be developed.

We are studying how aspects of nuclear compartmentalization affect DNA replication. We are developing a novel approach using single cell dynamic imaging to examine replication in real-time in living human cells. We are following the progression of DNA replication forks within telomeres and unusual DNA structures not having the standard double helical such as those present in chromosomal fragile sites.

**Long term interests:**

- Novel approaches to study regions of the genome that have been implicated in causing Alzheimer's disease and prostate cancer.
- Role of common Fragile sites in human cancer and cancer prone disorders such as Fanconi anemia.
- Regulation and reprogramming of DNA replication of human embryonic stem (ES) cells and induced pluripotent stem cells (iPS).
Triplet nucleotide expansion diseases and aging.

Current projects include a wide range of interests:

- Mechanisms leading to breaks at sites that result in chromosomal rearrangements frequently detected in Alzheimer’s disease and cancer cells.
- Understanding trinucleotide repeat expansion and telomere maintenance to gain insights into aging related disorders.
- Triplet nucleotide expansion diseases. The fragile X premutation expansion to 55 – 200 CGGs affects ~ one in 200 women resulting in serious fertility problems and ataxia.
- Study of human ES cell DNA replication dynamics. Understanding of replication programs to advance the availability of immunologically compatible hES cells for patients.

SELECTED PUBLICATIONS:

Key Words: Chromatin, epigenetics, transcription, proliferation, differentiation, leukemia

Our laboratory is interested in understanding the mechanisms controlling mammalian development and cell differentiation. We study the epigenetic functions of chromatin proteins and transcription factors in control of gene expression in embryonic stem cells, in red blood cells, and in Drosophila. Our approaches involve directed gene inactivation and transgenesis in mice and Drosophila. We also study control of proliferation and differentiation in red blood cell progenitors and in leukemia cells in which normal development is disrupted. Currently there are two major projects underway in the lab.

Role of H1 Linker Histones and Chromatin Remodeling Factors in Chromatin Structure, DNA Methylation, the Histone Code, Gene Expression and Development in Mice and Drosophila. Recent studies show that posttranslational modifications of core histones (H2A, H2B, H3, H4) (the Histone Code) play a very important role in control of gene expression. The H1 linker histones are more diverse than the core histones. Mice contain 8 H1 histone subtypes including differentiation-specific and tissue-specific subtypes, whereas Drosophila has only one type of H1. H1’s are thought to be responsible for the final level of packaging DNA into the compact chromatin structure but we know very little about their role in gene expression and development. We are studying the functional roles of H1 linker histones by inactivating (knocking-out) specific H1 genes in mice and the single H1 in Drosophila. We are also reintroducing mutant H1 linker histones into H1 depleted mouse cells and flies, to perform structure-function studies. We have also established a new role for H1 histone in DNA methylation, genomic imprinting and establishment of the histone code. We are also studying the chromatin remodeling factor that assembles H1 histone into chromatin.

Control of Proliferation and Differentiation in Normal and Leukemic Blood Cells: In this project we are investigating how cell proliferation and differentiation are coordinated in normal blood cell development and how this coordination is disrupted in leukemia. We have investigating the molecular mechanisms for the cross talk between these two cellular programs in normal and leukemic blood cells. Our studies are focused on the relationships between the master transcription factors that control blood cell development and the cell cycle regulators (cyclins, cyclin-dependent kinases (cdks), cdk inhibitors and RB) that regulate the cell division cycle proliferation. This project includes genome-wide approaches involving chromatin immunoprecipitation and high throughput sequencing (ChIP-Seq) and gene expression profiling by RNA-Seq.

Selected Publications:


FOLLOWING THE TRAVELS OF RNA

Our work is focused on the expression and travels of RNA within the cell: from the site of its birth to its ultimate biological destiny in the cytoplasm where it makes proteins in specific locations.

Our new technology, based on in situ hybridization allows us to visualize specific nucleic acid sequences within individual cells. Synthetic nucleic acid probes are labeled with fluorochromes. Subsequently these molecules are hybridized to the cell and detected using high resolution digital imaging microscopy.

We have developed imaging methodologies and algorithms capable of detecting a single RNA molecule within a cell. This enables the detection of specific nucleic acid molecules for comparison between normal or cancer cells. This method of molecular diagnosis is the clinical application of the technology. As an additional result of this approach, we have found specific RNA sequences located in particular cellular compartments. An example is the messenger RNA for beta-actin, which is located in the periphery of the cell where actin protein is needed for cell motility. These transcripts are not free to diffuse, and appear to be associated with a cellular matrix or skeleton from the moment of their synthesis through translation.

We are investigating how this spatial information is encoded within the gene and how the RNA transcript is processed within the nucleus and then transported to its correct compartment in the cytoplasm, resulting in asymmetric protein distribution.

RNA localization also occurs in yeast. During budding, a nuclear factor represses mating type switching asymmetrically, only in the daughter cell. This is because the factor is synthesized only in the bud because the mRNA was transported there by a motor, myosin. This discovery has provided a model by which to investigate the mechanisms responsible for moving RNA within the cell. For example, we have constructed genetically altered yeast and vertebrate cells in order to elucidate the sequences responsible for mRNA localization. A reporter gene can be "delivered" to a variety of cellular compartments by using specific sequences, or "zipcodes" from the mRNAs found in those compartments. These "zipcodes" consist of short sequences in the 3' untranslated region of the mRNA.

Recently we have developed technology that allows us to image RNA movement in living cells and tissues and characterize how the motors connect with and drive the RNA. Recent developments have allowed us to visualize transcription and RNA life cycle from birth to death in transgenic mice, including translation of single mRNAs.
Selected Publications:


Glycan Functions in Development, Spermatogenesis and Notch Signaling

Glycosylation is the most abundant and varied post-translational modification of proteins and is a critical factor in regulating their biological functions. The complement of glycans that may be produced by an organism is called the GLYCOME. Changes in glycans expressed on the cell surface occur during development and differentiation. Specific glycans on Notch receptors modulate signal transduction by Notch ligands. This is a novel paradigm of signal transduction whereby the transfer of a single sugar residue alters the ability of Notch receptors to signal. We are using cell-based glycosylation mutants, Notch signaling assays, glycosyltransferase gene knockout mice, and biochemical approaches including MALDI-TOF mass spectrometry, to identify biological functions of growth factor receptor and Notch glycans, and the underlying mechanisms by which glycans mediate biological events.

Notch receptors span the cell membrane. When a Notch ligand like Delta or Jagged on an apposing cell binds to a Notch receptor, it induces cleavage of Notch extracellular domain, followed by a second cleavage that releases Notch intracellular domain. The Notch intracellular domain goes to the nucleus and activates target genes that ultimately lead to a change in cell fate or cell growth control. Using a CHO glycosylation mutant that adds few O-fucose glycans to Notch extracellular domain, we showed that Notch signaling is markedly reduced when fucose is limiting. Using a panel of different CHO glycosylation mutants developed in this lab, we showed that inhibition of Notch signaling by the Fringe glycosyltransferase requires the addition of a Gal residue to O-fucose glycans on Notch. We are continuing to use Notch signaling assays to define the mechanisms of action of Fringe and other glycosyltransferases that modulate Notch signaling. We are also targeting glycosyltransferase genes that encode enzymes that modify Notch in the mouse, and generating Notch mutants that cannot accept an O-fucose glycan at a specific site in Notch. Mice lacking O-fucose in the ligand binding domain have defective T cell development and are being investigated for other immunological defects. Mice lacking the three Fringe activities are affected in T and B cell development. The most recent modification of Notch is by O-GlcNAc and we are now exploring its functions in the regulation of Notch signaling in mammals.

We are also investigating a novel inhibitor of complex N-glycan synthesis termed GnT1IP-L. It is expressed mainly in testicular germ cells in a highly regulated manner during spermatogenesis. Expression of this gene causes cells to bind strongly to Sertoli cells and we predict that it will be important for germ-Sertoli cell interactions necessary for spermatogenesis. We are testing this hypothesis by conditional deletion of the inhibitor in spermatogonia and also whole body knockout. We have found that complex N-glycans are essential for male fertility and are testing the hypothesis that they play an important role in spermatid/Sertoli cell interactions.

Finally, Chinese hamster ovary (CHO) cell glycosylation mutants developed in this laboratory are used by us and many laboratories for a variety of purposes including to develop new methods such as a novel approach to tracking glycan epitopes on the cell surface, a method to analyse glycans expressed and scRNA-seq termed sgRNA-seq, assays for Notch and growth factor signaling, and assays to characterize glycosylation mutations that cause rare congenital disorders of glycosylation (CDGs).
Selected References


A complete list of references can be found at https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40358567/?sort=date&direction=ascending
Molecular Regulation and Therapeutic Targeting of Pre-Cancerous and Cancer Stem Cells in Hematopoiesis and Leukemogenesis

Hematopoiesis maintains a life-long supply of the entire spectrum of highly specialized blood cells dependent on systemic needs. This process relies on a tightly regulated balance of self-renewal, commitment, and differentiation of a small number of pluripotent hematopoietic stem cells (HSC).

Recent experimental evidence has shown that acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS) arise from transformed immature hematopoietic cells following the accumulation of genetic and epigenetic changes in HSC and committed progenitors. These aberrations give rise to a highly diverse pool of pre-leukemic stem cells (pre-LSC), preceding the formation of fully transformed leukemia stem cells (LSC). Pre-LSC as well as LSC are characterized by a relative resistance to chemotherapy and thereby contribute to treatment failure. As a consequence, and despite the use of poly-chemotherapy and newer agents that transiently reduce the tumor burden, relapse continues to be the most common cause of death in most subtypes of AML and MDS. Defining the molecular characteristics, heterogeneity, and regulatory mechanisms governing pre-LSC and their subclonal diversity, dynamics, and progression to fully transformed LSC is critical to understanding the genesis of leukemia and to developing therapeutic strategies by which these cells can be eradicated.

Recent findings from our own group and others have demonstrated a critical role of key transcriptional regulators, chromatin-remodeling factors, and mediators of aberrant signaling in the genesis and function of pre-LSC and LSC in AML and MDS in mouse and human model systems.

The goal of our research is to delineate critical mechanisms in HSC that drive formation, progression, and therapeutic resistance of pre-LSC and LSC. To identify and functionally study implicated pathways we are utilizing stem and progenitor cell subsets isolated by means of multi-parameter high-speed fluorescence-activated cell sorting (FACS). We are using experimental tools including at the single-molecule and single-cell level, as well as molecular biological methods for forced expression or inactivation of molecular targets, followed by in vitro as well as in vivo assays for stem and progenitor cell functions including murine transplantation models. This allows for assessing the function of candidate mechanisms in normal and leukemic stem cells. We are studying murine genetic models as well as primary human samples from patients with pre-leukemic conditions and leukemia. Our studies aim at the development of targeted, pre-LSC- and LSC-directed therapies.

Project areas in the lab include:

- Mechanisms of leukemia pathogenesis at the (pre-leukemic) stem cell level
- Identification and study of novel molecular mechanisms and pathways governing normal and malignant hematopoiesis, including stem cell subclonal heterogeneity, dynamics, and competition (focusing on transcription and signaling, incl. single-molecule and single-cell studies)
- Development and preclinical testing of novel therapeutics targeting aberrant stem cells
- Translational computational biology (e.g. integrated analysis of WGS, scRNA-seq, ChIP-seq, CUT&TAG data etc.; including from longitudinally sampled, sorted / single stem cells from patients and mouse genetic models)
Selected publications:


Using CRISPR-mediated Chemical Genetics to Define Oncogenic Transcription Networks

The disruption of normal gene expression programs is a hallmark of all cancers. One common way in which this occurs is through chromosomal translocation that results in the generation of gain-of-function transcription factor fusion proteins. For example, PAX3-FOXO1, arises from the t(2;13) translocation, and is the defining feature of a subset of highly aggressive, pediatric rhabdomyosarcoma (RMS), while AML1-ETO arises from the t(8;21) translocation in acute myeloid leukemia (AML). Both of these translocations target transcription factors that regulate critical cell fate decisions, and both are thought to be the initiating event and an ideal therapeutic target in the cancer in which they arise. Thus, it is essential to understand how these oncogenic transcription factors alter transcriptional programs to drive cancer development.

Historically, our ability to understand how sequence-specific transcription factors rapidly and specifically alter transcriptional programs has been limited by a toolbox of very slow genetic and knockdown strategies that take days to weeks before transcription factor activity can be assayed. Therefore, while direct transcriptional effects occur within minutes to hours, these models take days to establish, which results in the detection of secondary and/or compensatory transcriptional changes that often mask the direct/immediate effects of transcription factor disruption. In order to overcome these technical limitations, we use CRISPR-mediated genome editing to introduce degron tags into endogenous transcription factor loci. This chemical-genetic approach allows rapid transcription factor degradation (minutes to hours) following PROTAC (e.g. dTAG-47, see figure above) treatment, and effectively collapses the timeframe for assaying transcriptional changes, chromatin states, and genome-wide transcription factor occupancy from days to hours. We also incorporate proteomics-based methods to identify associated protein complexes and cooperating transcription factors. Combined, these approaches are allowing us to define the mechanism of action of specific oncogenic transcription factors (e.g. AML1-ETO and PAX3-FOXO1). Moreover, we aim to address fundamental questions in the transcription field including how oncogenic transcription factors are influenced by and exert influence over the chromatin landscape, how multiple sequence-specific transcription factors cooperate to fine-tune gene expression, and how enhancer activity functions to control gene transcription.

Selected Publications:


Complete publication list available at:

MECHANISMS OF STEM CELL AGING AND TRANSFORMATION

Key Words: Hematopoietic stem cells, cell fate determination, hematopoietic malignancies.

Hematopoietic stem cells (HSC) maintain multi-lineage blood formation throughout our lifetime. Balancing stem cell regeneration and differentiation commitment to produce mature blood cells is quintessential for a healthy hematopoietic system. Dysregulation of such HSC fate determination processes can lead to loss of immune function, bone marrow failure, and malignant transformation during aging. Up to date, very little is known about the molecular events driving age-related HSC changes and how they contribute to disease.

Understanding age-associated molecular alterations will not only uncover fundamental mechanisms guiding function of HSC, but may also allow for therapeutic intervention to “rejuvenate” aged hematopoietic systems and possibly even prevent age-associated hematopoietic diseases. **Our mission is to clarify the central mechanisms establishing and guarding sustained hematopoietic stem cell function, particular those that drive leukemogenesis, if disrupted.** We develop innovative genetic mouse models, use *ex vivo* and *in vivo* primary mouse and human stem cell assay systems, exploit lentiviral gene transfer, and apply state-of-the-art molecular biology and next generation sequencing techniques.

**Identification of novel molecular safeguards of adult and cancer stem cells**
Teamed-up with Dr. Ana Maria Cuervo (Dept. of Developmental & Molecular Biology), leader in highly precise protein degradation and aging biology, we have recently discovered an important role for chaperone-mediated autophagy in the maintenance of functional HSC (*Dong et al., Nature 2021*). Current efforts investigate the role of this and other stress-related molecular defense mechanisms in leukemic stem cell evolution and maintenance.

**The labile iron pool as a rheostat for stem cell function**
Our recent work has uncovered a key role of the amount of readily accessible intracellular iron (termed labile iron pool, LIP) in instructing HSC self-renewal (*Kao et al., STM 2018*). We have been investigating the precise molecular mechanism of action, particularly focusing on metabolic and non-enzymatic molecular pathways relying on iron – a completely uncharted territory for healthy as well as leukemic stem cells.

**Gene expression program erosion in aging stem cells and leukemia**
Our past work has demonstrated a causative role of even minimal dosage alterations of a key transcription factor instructing hematopoiesis, PU.1, observed in hematopoietic stem cell aging to myeloid leukemia evolution (*Will et al., Nat Med 2015*). Our current efforts focus on understanding (1) how such slight deviations from optimal PU.1 dosage lead to the erosion of PU.1-dependent gene expression programs, and (2) how PU.1 gene expression networks functionally cooperate with age-associated inactivation of epigenetic regulators (e.g. TET2 and DNMT3A).

**Improving stem cell-directed therapies**
We are actively engaging with commercial research partners to test and evaluate novel therapeutic options for patients with hematologic malignancies (*Will et al., Blood 2012; Kao et al., STM 2018; Shastri et al., JCI 2018*).
Selected Publications

Full list of publications can be found at:


Transcription Regulation and Cell Signaling Control in Normal B/T Cells and Lymphomas

Molecular pathogenesis of lymphomas situates at the crossroad of lymphocyte development, cancer genetics, transcription regulation, and cell signaling. Thus, we constantly draw upon the most recent advances in these fields to address mechanism questions that are related to lymphoma initiation and development. As each lymphoma entity often corresponds to a specific B/T cell activation/differentiation state that is phenotypically “frozen” by the malignant transformation process, our lymphoma-related studies also provide valuable insights to the regulatory mechanisms that govern the normal immune system. Our research has three major goals: to better understand mature B and T cell development in molecular terms, to decipher how this process is perturbed during lymphomagenesis, and to develop mechanism-based novel therapies to improve patient outcome.

The germinal center (GC) response is a very important B cell development stage that has the unique property of generating high affinity antibodies and B cell memory. Because dysregulated GC responses contribute to the development of B cell lymphomas and autoimmune diseases, in-depth understanding of the control mechanisms governing the GC response has both immunological and clinical implications. GCs are dynamic and specialized structures in the secondary lymphoid organs where the B cell genome is subject to two types of genetic alterations catalyzed by AID (activation induced cytidine deaminase), e.g. Ig class switch recombination and somatic hypermutation. Prior to their GC exit, B cells bearing mutated surface Ig molecules undergo positive and negative selections through interaction with two other types of cells in the GC, e.g. follicular dendritic cells follicular T helper (Tfh), and T follicular regulatory (Tfr) cells. Only the fittest B cells are licensed to terminally differentiate into memory or plasma cells. At the single cell level, the acquisition and termination of GC phenotype is the coordinated transcriptional response to various extracellular and intracellular stimuli; yet the precise sequence and nature of events that orchestrate this process is incompletely understood. We are particularly interested in the roles of two transcriptional factors, BCL6 and STAT3, both are known to play pivotal roles in fate specification and function of GC B cells, Tfh, and Tfr cells.

Our studies over the past 25 years have revealed novel mechanisms that govern the expression and activity of BCL6, and demonstrated the importance of functional interactions between BCL6 and several cell signaling pathways including RhoA, NF-κB, and Jak/STAT3. In recent years, a central focus of our B cell lymphoma work was on the IL-6/Jak/STAT3 signaling pathway. With the help of our collaborators, we characterized expression regulation of STAT3, cause and consequences of its aberrant activity in diffuse large B-cell (DLBCL) development and therapeutic response. Functional contribution of this pathway to normal plasma cell maturation was also investigated.

Since three years ago, our work has taken on a new focus, i.e. the pathogenesis and immunologic features of adult T-cell leukemia/lymphoma (ATLL). ATLL is a rare CD4 T cell neoplasm, endemic in the Japanese, Caribbean and Latin American populations. It arises in HTLV-
1 carriers and is an extremely aggressive cancer with a dismal outcome and lack of effective therapies. In a recently study published in Blood, we demonstrate that ATLL patients diagnosed in North American (NA-ATLL) have a distinct genomic landscape compared to the Japanese cohort (J-ATLL). In particular, NA-ATLL is characterized by a much higher frequency of prognostic epigenetic mutations and is targetable preclinically with DNA de-methylation drugs. Taking advantage of the fact that the Montefiore Medical Center follows one of the largest groups of ATLL patients in the U.S, we have built up a NA-ATLL Biobank, generated and characterized a number of novel NA-ATLL cell lines and PDX models to support our laboratory studies.

We are currently pursuing the following research questions:
1. Can the therapeutic outcome of the ABC subtype of DLBCLs be improved by manipulating the endogenous redox homeostasis of the lymphoma cells?
2. How does BCL6 contribute to the pathogenesis and transcription programs of NA-ATLL?
3. What is the genetic and clonal evolution basis that underlies the profound chemo-resistance of ATLL?
4. Explore novel, targeted therapies for NA-ATLL.

Selected Publications:


